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Sex Hormone Changes Induced by the Parasite Lead to Feminization of the Male Host in Murine *Taenia crassiceps* Cysticercosis

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Female mice are more susceptible to *Taenia crassiceps* (TC) infection than males. However, after a month parasite load increases massively in both genders reaching thousands of parasites per host. The possibility of hormonal changes in the infected mice was envisaged. Sex hormones levels were assayed after different periods of infection, the parasites present in the peritoneal cavity were collected and gonads, uterus and seminal vesicles were weighed. In male mice, serum estradiol increased to levels 200 times their normal values whilst those of testosterone decreased 90% relative to controls. The weight of seminal vesicles was significantly diminished. Infected female mice also showed a slight increase in estrogen blood levels after 8 weeks of infection and the weight of the uterus was significantly increased relative to controls. Serum estradiol and testosterone were almost undetectable after gonadectomy. Cytokines such as IL-6 are capable of stimulating aromatase activity and we found that splenocytes from infected mice produced amounts of IL-6 higher than control as measured by ELISA. In conclusion *T. crassiceps* infection triggers a feminization process in the infected hosts. The gonads are required for the parasite to induce higher estrogen synthesis. IL-6 could be involved in the immunoendocrine mechanism used by the parasite to maintain a highly permissive environment for its rapid growth.

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INTRODUCTION

The sexual dimorphism that exists in the normal immune response and in many autoimmune diseases strongly suggests that a linkage between the immune and the reproductive endocrine system exists [1, 2].

Endocrinological, reciprocal interactions between host and parasite are receiving increased attention as influential in parasite success [3]. For instance *Taenia taeniformis* is known to alter reproduction in rats by somehow interfering with the normal functions of sex steroids [4]. The reproduction of *Brugia pahangi* and *Dirofilaria immitis* is modulated by ecdysteroids [5]. Sexual changes in body morphology as well as sex-related behavioral changes have been observed in crabs when parasitized with Rhizocefalan [6] through mechanisms that are still obscure and could involve changes in the hormonal pattern of the host. Sex preferences in several parasite diseases are well known [7–14].

Experimental murine cysticercosis caused by *Taenia crassiceps* has been used to explore the role of biological factors involved in host susceptibility. Several factors, such as genetics (major histocompatibility complex) and immune status (vaccinations) affect parasite growth [15, 16]. Furthermore, susceptibility to *T. crassiceps* infection in mice is associated with sex: in early infections females carry larger parasite loads than males although, later on, males also become massively parasitized [15]. The sluggish massive colonization of male mice by *T. crassiceps* suggested a sex hormone change in the host induced by the parasite resulting later-on

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in an increased susceptibility to the infection. To test the hypothesis we measured the level of major sex hormones and the weights of their target organs in chronically parasitized male and female mice. Gonadectomies, and androgen reconstitution, were performed to identify the specific organs and hormones involved in the host–parasite interactions. IL-6 was also studied, as a possible candidate mediating hormonal changes [17] in infected mice [18].

MATERIALS AND METHODS

Mice

Male and female Balb/c inbred mice were bred in our animal facilities by the "single-line breeding system" over 20 generations, starting with original stock from Jackson Labs in 1982, and were fed Purina's Diet 5015 *ad libitum*.

Parasites and experimental infections

The fast-growing ORF strain of *T. crassiceps*, isolated by Freeman in 1962 [19], was used for mice infection in all experiments and was supplied by Dr B. Enders (Behringwerke, Marburg, Germany) in 1986. Since then the parasites have been maintained in female Balb/c mice by i.p. sequential inoculation of metacestodes in their peritoneal cavity [19]. Larvae for experimental infection were obtained from female donor mice infected 3–6 months before. Ten small (approx. 2 mm diameter) non-budding *T. crassiceps* larvae were suspended in 0.3 ml PBS (0.15 M NaCl, 0.01 M sodium phosphate buffer, pH 7.2) and injected i.p. into each 42-day-old mouse using a .25 gauge needle. Mice were sacrificed (etherization) in different periods after infection and all the cysts found inside the peritoneal cavity were counted. A complete parasite count was performed visually in each mouse after sacrifice by collecting all parasites present in the peritoneal cavity after thoroughly rinsing it with phosphate buffered saline (NaCl 0.15 M, Na_2PO_4 0.2 M, $NaHPO_4$ 0.1 M). An autopsy followed, including gonads and uterus' or seminal vesicles' weight determinations. Organs were placed in 10% formalin for ulterior light-microscopy examination. In this form of disease the parasites do not migrate to another location in the host. Heavily parasitized mice show enlarged abdomens but the disease does not seem to alter other features nor normal body weights (discounting the weights of parasites, which may actually equal that of the host, approx. 25 g).

Treatment procedures

Gonadectomies were surgically performed under ether anesthesia on 4-week-old mice of both sexes. Mice were then allowed a 1 week recovery period before inoculation with parasites. In reconstitution experiments, androgen was administered 7 days after gonadectomy with either testosterone or 5α-dihydrotestosterone (0.5 mg in 3 weeks release pellets, Innovative Research of America, Toledo, OH). After another 7 days mice were inoculated with parasites as described above. The effects of the androgens upon parasite loads were measured 8 weeks after infection.

Hormone measurements

Blood for estradiol and testosterone determinations was collected *in vivo* by retrocular venous puncture performed in mice under ether anesthesia. After incubation for 18 h at 4°C the blood clot was centrifuged and serum was separated. Steroids were ether-extracted and solubilized in the phosphate buffer used for radio immunoassay (RIA) [20, 21]. The concentrations of estradiol and testosterone were determined by RIA, each in duplicate. The antisera were all supplied by ICN Biomedical Inc. (Costa Mesa, CA). The estradiol antiserum cross-reacts 2.5% with oestrone and 1.3% with 17α-estradiol, and the testosterone antiserum cross-reacts 18.8% with 5α-dihydrotestosterone (DHT) and 3% with 5α-androsterone-3α-17β-diol. Tritiated ligands 1,2,6,7[^{3}H]testosterone and 1,2,4,8[^{3}H]estradiol were supplied by New England Nuclear (Boston, MS). RIA data were analyzed by the logit/log regression analysis as described [20, 21].

Interleukin-6 assays

IL-6 measurement in stimulated lymphocytes [22] and in serum were performed by ELISA using a kit of Pharmingen (San Diego, CA). Lymphocytes were obtained from the spleens of 4 and 32 weeks parasitized animals and their respective controls. Cells were cultured in the presence or absence of concanavalin-A, and after incubation (37°C, 48 h) IL-6 was measured in the culture medium by ELISA, following the kits' instructions.

RESULTS

Parasite load

A few days after inoculation cysticerci begin active asexual reproduction by budding in one of the poles of their oblong cystic structure [Fig. 1(b)]. A few months later they count in the hundreds or thousands, reaching masses that may equal those of the host [Fig. 1(a) illustrates the differences in abdomen size between a normal mouse and its parasitized litter-mate after 6 months of infection]. *T. crassiceps* cysticerci initially grow faster in females than in males but, in late infections, males are also burdened by huge parasite loads (Table 1). Health of the massively parasitized mice is not seriously affected in laboratory conditions nor are there macroscopic or microscopic signs of serious illness or malnutrition.

Testosterone and estradiol serum levels

Figure 2 shows the results obtained measuring serum levels of testosterone and estradiol in male and female mice during the course of cysticercus infection. The

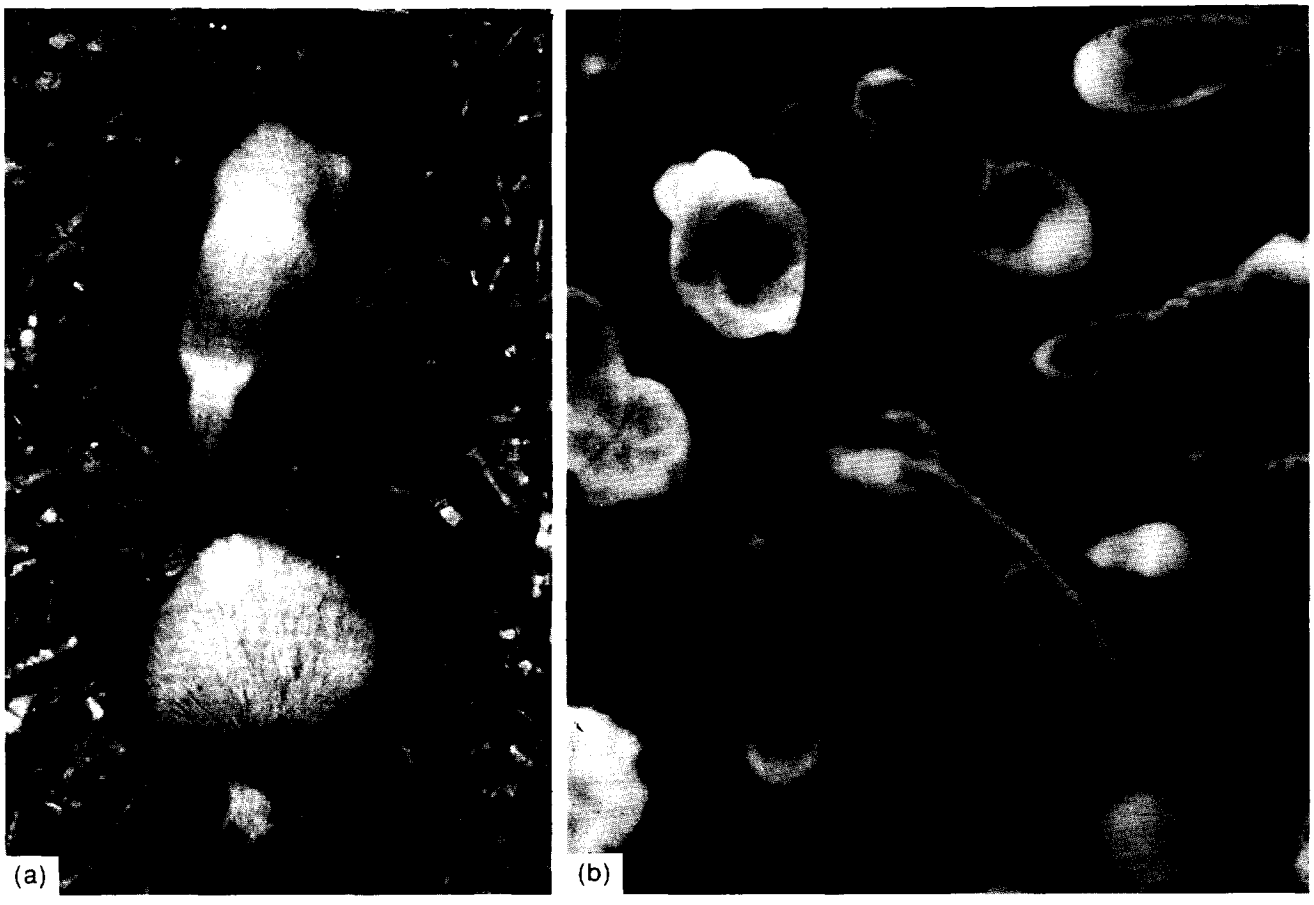

Fig. 1. Main features of experimental murine cysticercosis. (a) This figure illustrates the differences in abdomen size between a normal mouse and its parasitized litter-mate after 6 months of infection. (b) Asexual reproduction of parasites begins a couple of weeks after injection into the peritoneal cavity of mice.

growth of the parasite in the abdominal cavity of males is followed by an increment in blood estradiol that increases to levels 200 times their normal values whilst that of testosterone decreases 90% relative to control [Fig. 2]. Infected females also show increased blood levels of estradiol at 8 and 16 weeks after infection [Fig. 2].

Weights of uterus and seminal vesicles

The weight of the uterus in infected females is increased relative to controls after 4 weeks of infection and remains hypertrophied at 16 weeks of infection (Fig. 3, bottom). The weight of the seminal vesicles is significantly reduced in parasitized males after 4 weeks of infection and differences continued being evident at 8 and 16 weeks post infection (Fig. 3, top).

Effects of gonadectomy

The origin of the increment in blood estradiol was examined by simultaneously studying parasite loads and levels of sex steroids 4 and 8 weeks after experimental infection in both control and gonadectomized male and female mice, a time of infection when major endocrinological alterations were clearly detectable in the previous experiments with intact mice. After gonadectomy serum estradiol and testosterone decreased

Table 1. Time-course of infection in male and female mice. Initially T. Crassiceps cysticerci grows faster in females than in males but, in late infections, males are also burdenend by huge parasite loads

	Infection time (days)									
	1	2	3	5	8	15	20	30	60	150
Female	0.8 ± 0.4	1.8 ± 1.0	3.1 ± 0.5	0.6 ± 0.3	5.9 ± 0.5	10.8 ± 1.0	25.7 ± 4.5	131.8 ± 5.1	582.1 ± 40.4	2642.9 ± 171.8
	$n = 9$	$n = 5$	$n = 19$	$n = 14$	$n = 53$	$n = 20$	$n = 27$	$n = 371$	$n = 34$	$n = 18$
Male	0.4 ± 0.2	0.5 ± 0.2	2.1 ± 0.5	0.0 ± 0.0	2.2 ± 0.3	6.3 ± 0.8	7.6 ± 1.4	30.0 ± 1.9	247.5 ± 49.9	1144.3 ± 130.3
	$n = 9$	$n = 6$	$n = 19$	$n = 14$	$n = 53$	$n = 20$	$n = 28$	$n = 329$	$n = 28$	$n = 19$

Data represent mean $\pm$ SE of individual parasite loads of a total of 573 female and 525 male infected, and otherwise untreated mice, registered in the database, over a number of experiments performed at different times.

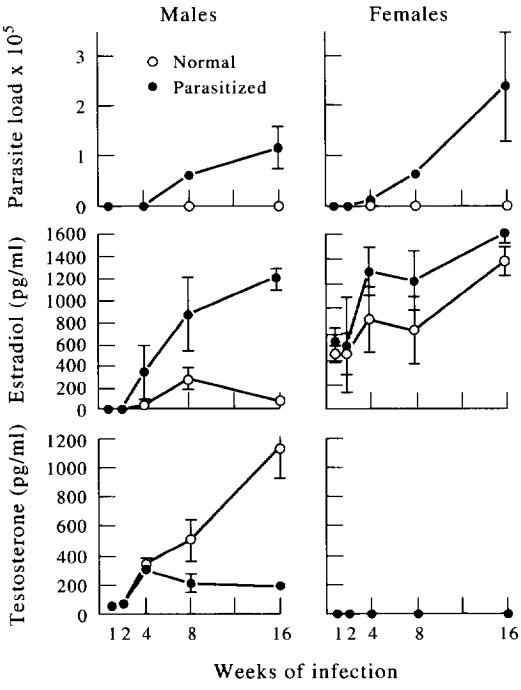

Fig. 2. Impact of cysticercosis in levels of sex-steroids. Modifications in individual parasite counts and in estradiol and testosterone serum concentrations of male and female mice after different weeks of intraperitoneal infection. Each symbol in the graph represents the mean ± 1 SD of the response variable as obtained from at least 5 mice in each time of infection, and by duplicate in each mouse for sex steroid assessments. The experiment was performed a second time in the same conditions with essentially the same results.

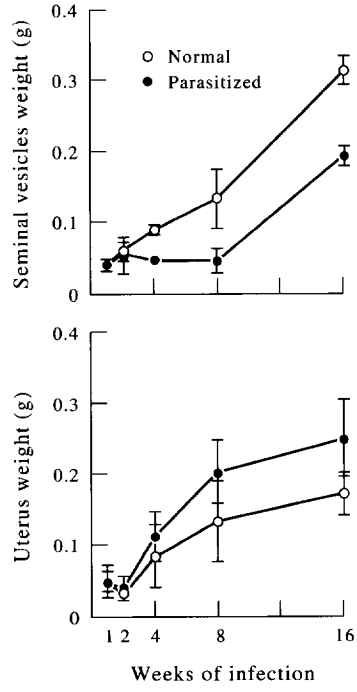

Fig. 3. Impact of cysticercosis in sex-steroids' target organs. Wet weights (average ± 1 SD; $n = 5$ for each time) of sex steroids' main target organs of mice in the course of intraperitoneal infection with *T. crassiceps* cysticerci. The weight of seminal vesicles are reduced relative to control mice after 4 weeks of infection ($P < 0.01$), whereas the weight of uterus increased significantly in infected females after 8 weeks of infection ($P < 0.05$). Statistics were calculated by analysis of variance of the multifactorial design with gender, weeks of infection and infected or not, as treatment variables; and weight of organs as response variable, and replicates (mice) = 5 in each treatment combination. Data express the mean ± 1 SD.

to almost undetectable levels in both male and female mice (not shown). However, 8 weeks after infection, the parasite loads in gonadectomized males were greater than those of intact controls and smaller in gonadectomized female mice than those of controls (Table 2).

Effects of androgen reconstitution

To test the androgen's parasite restrictive activities, testosterone and DHT were administered to gonadectomized male and female mice as described. Both testosterone and DHT decreased parasite load, although the effect of androgens on males is less significant (Table 2). Testosterone decreased parasite load by 32% in females ($P < 0.01$) and by 32% in males ($P < 0.01$), while DHT decreases 34% parasite load in females ($P < 0.01$) and 27% in males ($P < 0.01$).

IL-6 assays

Table 3 and Fig. 4 show the results obtained in this set of experiments as performed in intact control and parasitized male and female mice. Serum levels of IL-6 are significantly increased in parasitized male and female mice and so is IL-6 production in the cultured medium of lymphocytes from parasitized mice 4 and 32 weeks after infection.

DISCUSSION

The measurement of sex steroids in infected male and female mice indicates that *T. crassiceps* triggers a feminization process in both genders. Feminization is most outstanding in male mice where blood estradiol increases to levels 200 times their normal values, roughly similar to those of normal females, whilst those of testosterone decrease 90% relative to control. The weight changes in target organs sex hormones (seminal vesicles and uterus) support that the hormone changes in the infected host are physiologically relevant.

Table 2. Parasite load 8 weeks after infection with T. Crassiceps in intact (P) and gonadectomized (Gx) male and female mice. Some animals were implanted with testosterone (T) or dehydrostestosterone (DHT) release pellets

n		Female	Male
10	P	998.90 ± 519.22	188.90 ± 93.32
10	GxP	276.80 ± 50.98	302.30 ± 103.39
10	GxP + T	186.70 ± 50.01	205.40 ± 28.64
10	GxP + DHT	182.10 ± 57.73	221.67 ± 81.06

Data represent mean number of parasites/mouse ± SD.

Table 3. Serum IL-6 concentration in male and female mice after 4 and 8 weeks of infection

	Female	Male
4 weeks		
Control	25.01 ± 14.44	0.964 ± 0.556
Parasitized	110.70 ± 66.05	45.88 ± 20.52
8 weeks		
Control	119.10 ± 76.60	82 ± 57
Parasitized	136.76 ± 90.406	165.13 ± 12.49

IL-6 (pg/ml) was determined by ELISA in each serum by triplicate.
Data represent mean ± SD.

How does the cysticercus manage to feminize and demasculate its host? Perhaps the cysticercus favors its growth by producing its own estrogens (as *Spirometra mansonoides* produces a kind of growth hormone when infecting rodents [23] and many other parasites produce ecdysone [3]), or somehow stimulates the host's endocrine system towards abnormal oestrogen synthesis. Serum estradiol and testosterone decreases to almost undetectable levels in infected gonadectomized mice, thus indicating that the host's gonads are required for the parasite to induce high estradiol synthesis in both sexes. However, the absence of estrogens does not prevent the growth of parasites in both genders demonstrating that although estradiol favors *T. crassiceps* development [24–26] it is not indispensable for parasite growth. Other gonad-associated factors in the control of parasite growth and a more intricate strategy in the parasite's activity has to be considered. Perhaps the low androgen levels are the principal feature in this intriguing puzzle. Because the parasite-loads of males increased upon castration, even in the absence of estradiol, we suspected that androgens are inhibitory, a hypothesis that was confirmed when testosterone and DHT decreased parasite loads in gonadectomized male and female mice.

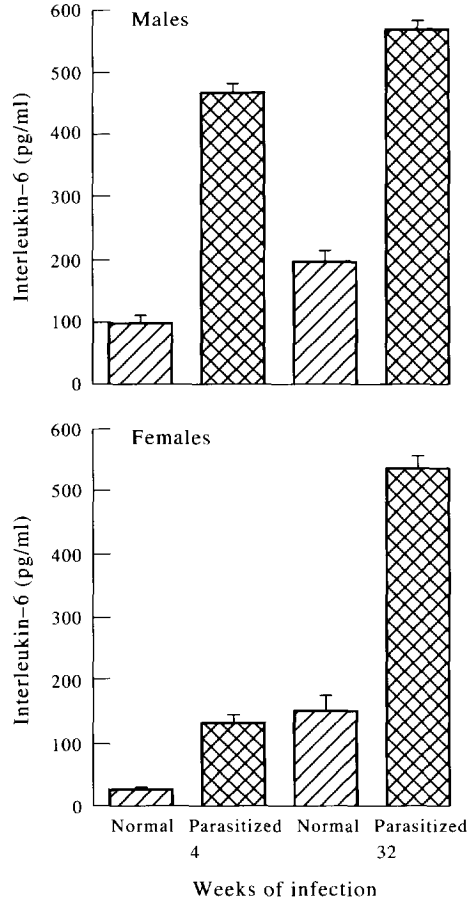

Fig. 4. Production of IL-6 by lymphocytes. IL-6 production was measured in the culture medium of spleen lymphocytes of male (top) and female (bottom) mice after being cultured for 48 h in the presence of concanavalin-A. Data represent mean ± 1 SD of an experiment made by triplicate at 4 and 32 weeks of infection.

At this point we conclude that sexual differences in parasite loads in intact mice favor females because estradiol promotes and androgen restricts the growth of *T. crassiceps* cysticerci. We further deduce that the cysticerci eventually grow as massively in male as in female hosts because it induces an estrogenization and deandrogenization process in the male host by disabling the normal hormonal function of the testis.

A simple strategy of the cysticercus to achieve these high levels of estradiol and low levels of testosterone in the male host would involve stimulation of the aromatase pathway, the enzyme responsible for the conversion of testosterone to estradiol [27]. This possibility would also be consistent with the preference of the parasite towards females in the normal hosts, in which through an active aromatization process high levels of estradiol are naturally being produced.

Speculations about an enhanced aromatase activity requires revision of the factors involved in its modulation. For instance, IL-6 has been shown to stimulate aromatase activity in breast cancer cells [17] and to be deeply altered in a variety of infections [18]. Thus, we measured the production of this cytokine in parasitized mice. The results shown in this paper demonstrate an important increment in IL-6 concentration of blood and culture medium of lymphocytes from parasitized mice, that could be a factor involved in the feminization process that develops in infected mice.

The cunning hormonally based strategy employed by *T. crassiceps* to establish an unseemly mass of foreign tissue in an initially normal comparatively resistant male mouse may be of interest for consideration in other chronic and massive host-parasite confrontations. Host feminization by parasitic disease illustrates the plasticity of sexual phenotype in response to infections involving the immune system and, by endangering the reproductive capacity of the host, poses novel forms of affecting the evolution of both host and parasite other than the prey/predator approach.

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